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(57) Abstract

A linear (i.e., non-cyclic) analog of biologically active amphibian bombesin or mammalian gastrin-releasing peptide (GRP) having an active site and a binding site responsible for the binding of the peptide to a receptor on a target cell. Cleavage of a peptide bond in the active site of naturally occuring bombesin or GRP is unnecessary for *in vivo* biological activity. The analog has one of the following modifications: (a) a deletion of an amino acid residue within the active site and a modification of an amino acid residue outside of the active site, (b) a replacement of two amino acid residues within the active site with a synthetic amino acid, a β-amino acid, or a γ-amino acid residue, or (c) a non-peptide bond instead of a peptide bond between an amino acid residue of the active site and an adjacent amino acid residue.

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THERAPEUTIC PEPTIDES

Background of the Invention

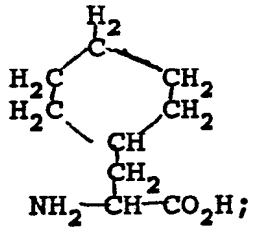
This invention relates to therapeutic peptides useful, e.g., for treatment of benign or malignant proliferation of tissue, for gastrointestinal disorders, and for diabetes.

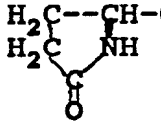
The amphibian peptide bombesin, pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (Anastasi et al., Experientia 27:166-167 (1971)), is closely related to the mammalian gastrin-releasing peptides (GRP), e.g., the porcine GRP, H_2N-Ala-Pro-Val-Ser-Val-Gly-Gly-Gly-Thr-Val-Leu-Ala-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-(NH_2) (McDonald et al., Biochem. Biophys. Res. Commun. 90:227-233 (1979)) and human GRP, H_2N-Val-Pro-Leu-Pro-Ala-Gly-Gly-Gly-Thr-Val-Leu-Thr-Lys-Met-Tyr-Pro-Arg-Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met (NH_2). Bombesin has been found to be a growth factor for a number of human cancer cell lines, including small-cell lung carcinoma (SCLC), and has been detected in human breast and prostate cancer (Haveman et al., eds. Recent Results in Cancer Research - Peptide Hormones in Lung Cancer, Springer-Verlag, New York:1986). A number of these cancers are known to secrete peptide hormones related to GRP or bombesin. Consequently, antagonists to bombesin have been proposed as agents for the treatment of these cancers.

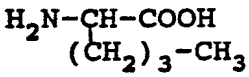
Cuttitta et al. demonstrated that a specific monoclonal antibody to bombesin inhibited in vivo the growth of a human small-cell lung cancer cell line xenografted to nude mice (Cuttitta et al., Cancer Survey 4:707-727 (1985)). In 3T3 murine fibroblasts which are responsive to the mitotic effect of bombesin, Zachary

and Rozengurt observed that a substance P antagonist (Spantide) acted as a bombesin antagonist (Zachary et al., Proc. Natl. Acad. Sci. (USA), 82:7616-7620 (1985)). Heinz-Erian et al. replaced His at position 12 in bombesin with D-Phe and observed bombesin antagonist activity in dispersed acini from guinea pig pancreas (Heinz-Erian et al., Am. J. of Physiol. 252:G439-G442 (1987)). Rivier reported work directed toward restricting the conformational freedom of the bioactive C-terminal decapeptide of bombesin by incorporating intramolecular disulfide bridges; however, Rivier mentioned that, so far, bombesin analogs with this modification fail to exhibit any antagonist activity (Rivier et al., "Competitive Antagonists of Peptide Hormones," in Abstracts of the International Symposium on Bombesin-Like Peptides in Health and Disease, Rome, Italy (October, 1987).

Abbreviations (uncommon):

cyclohexyl-Ala =  (cyclohexyl alanine) identifying group;

pGlu =  (pyroglutamic acid);

Nle =  (norleucine)

Pal = 3-pyridyl-alanine

β-leu = β - homoleucine

γ-leu = gamma - homoleucine

D-Cpa = D-p-chlorophenylalanine

HyPro = hydroxyproline

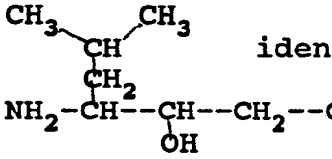
Nal = naphthylalanine

Sar = sarcosine

F_5-Phe = penta-fluoro-Phenylalanine

Sta (statine) =

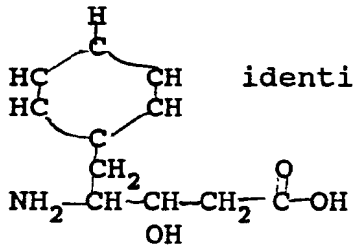
(3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid, and has the chemical structure:



identifying group

AHPPA =

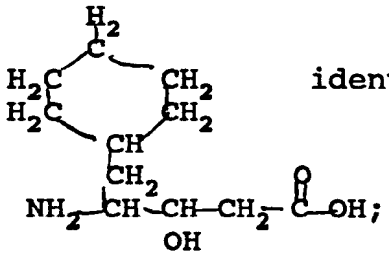
(3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid, and has the chemical structure:



identifying group

ACHPA =

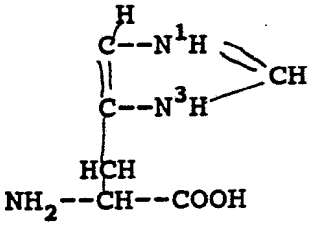
(3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid and has the chemical structure:



identifying group

R = right (D) configuration; S = left (L) configuration; racemate = equal mix of R and S

1-methyl-His; 3-methyl-His = methyl (CH_3) group on nitrogen at positions 1 or 3 of Histidine:



Summary of the Invention

In general, the invention features a linear (i.e., non-cyclic) analog of biologically active mamalian gastrin-releasing peptide (GRP) or amphibian bombesin, having an active site and a binding site responsible for the binding of the peptide to a receptor on a target cell; cleavage of a peptide bond in the active site of naturally occurring bombesin or GRP is unnecessary for in vivo biological activity. The analog has one of the following modifications: (1) a replacement of two amino acid residues within the active site with a β-amino acid, or a γ-amino acid residue, (2) a non-peptide bond instead of a peptide bond between an amino acid residue of the active site and an adjacent amino acid residue, (3) a deletion of one carboxy-terminal amino acid of the peptide which is accompanied by the carboxy-terminal amino acid being of the ester or amide form, or (4) alkylation of the amide bond between the penultimate and the ultimate carboxy-terminal amino acid.

In preferred embodiments the analog is capable of acting as a competitive inhibitor of the naturally occurring peptide by binding to the receptor and, by virtue of one of the modifications, failing to exhibit the in vivo biological activity of the naturally occurring peptide.

The locations of the modifications that give rise to antagonists are determined by the location of the active site in the naturally occuring peptide. For example, the linear peptides for which introduction of a

non-peptide bond between the carboxyl terminal and adjacent amino acid residues, or the replacement of the natural carboxyl terminal and adjacent amino acid residues with a β-, or γ- amino acid residue, or the deletion ("des") of the C-terminal amino acid residue are useful in creating or enhancing antagonist activity are those in which activity is associated with the two C-terminal amino acid residues of the amino acid chain. Similarly, where the active site is located in the amino terminal portion of the naturally occuring peptide, the corresponding analogs of the invention will possess modifications in their amino terminal portions.

In preferred embodiments the active site includes at least one amino acid residue located in the carboxyl terminal half of the naturally occurring biologically active peptide and that amino acid residue is located in the carboxyl terminal half of the linear peptide.

In preferred embodiments the binding sites includes at least one amino acid residue located in the amino terminal half of the naturally occurring biologically active peptide and that amino acid residue is located in the amino terminal half of the linear peptide.

Modifications can be introduced in a region involved in receptor binding, or in a non-binding region. Preferably, analogs of the invention are 25% homologous, most preferably, 50% homologous, with the naturally occurring peptides.

The analogs of the invention may have one of the modifications given in the generic formula given below; either a non-peptide bond instead of a peptide bond between an amino acid residue of the active site and an adjacent amino acid residue; or a synthetic amino acid, e.g. a statine, an AHPPA, or an ACHPA, a β-amino

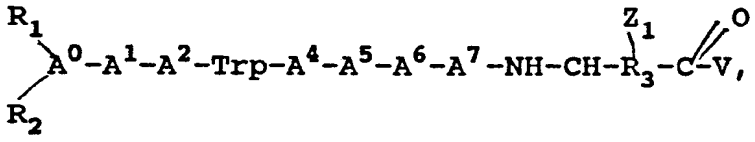
acid, or a γ-amino acid residue in place of two natural amino acid residues; or a deletion of the C-terminal amino acid residue, accompanied by the addition of a substituent on the actual C-terminal group and the presence of an N-terminal residue that is not the natural N-terminal amino acid residue of the peptides from which the analogs are derived. (Statine, AHPPA, and ACHPA have the chemical structures defined above. Where statine is used herein, AHPPA or ACHPA may also be used.)

By non-peptide bond is meant that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon, i.e., CH_2-NH; or, less preferably that CO-NH is replaced with any of CH_2-S, CH_2-O, CH_2-CH_2, CH_2-CO, or CO-CH_2. (A detailed discussion of the chemistry of non-peptide bonds is given in Coy et al. (1988) Tetrahedron 44,3:835-841, hereby incorporated by reference, Tourwe (1985) Janssen Chim. Acta 3:3-15, 17-18, hereby incorporated by reference, and Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, (B. Weinstein, ed.) M. Dekker, New York and Basel, pp. 267-357, hereby incorporated by reference.)

One modification of the naturally occurring peptide to create an antagonist is of the amino terminal end of the molecule, such as those described for the amino terminal positions in the generic formula below; for example, the N-terminal amino acid residue, which is A^0 or, if A^0 is deleted, is A^1 or, if A^0 and A^1 are deleted, is A^2 below, may be an aromatic D-isomer, or may be an alkylated amino acid residue. (Where "D" is not designated as the configuration of an amino acid, L is intended; furthermore, where R or S is designated in the generic formulae, the D (R) or L (S) form of an amino

acid may occur at any position.)

The therapeutic peptide includes between seven and ten amino acid residues, inclusive, and is an analog of one of the following peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide (GRP); and (c) the ten amino acid carboxy-terminal region of amphibian bombesin. The therapeutic peptide is of the following formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

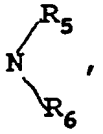
A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

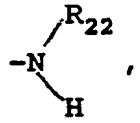
A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

wherein R_3 is $CHR_{20}-(CH_2)_{n1}$ (where R_{20} is either of H or OH; and n1 is either of 1 or 0), or is deleted, and provided that where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 is the identifying group of any of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, p-X-Phe (where X = H, F, Cl, Br, NO_2, OH, or CH_3), F_5-Phe, Trp, Cys, Met, Pro, HyPro, cyclohexyl-Ala, or β-nal; provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, Z_1 can only be F_5-Phe; and V is either

OR_4, or  ,

where R_4 is any of C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl, and each R_5, and R_6, independently, is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, lower acyl, or,

 ,

where R_{22} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or lower acyl; provided that, when one of R_5 or R_6 is $-NHR_{22}$, the other is H; provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that

each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

In preferred embodiments, the therapeutic peptide has the formula

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;

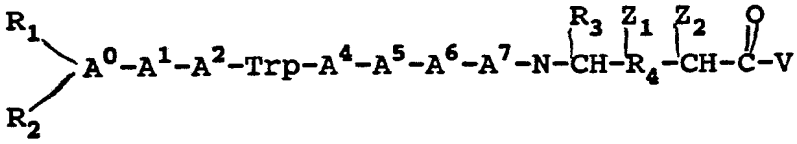
A^7 = His;

and, where A^1 is F^5-D-Phe or A^6 is N-methyl-D-Ala, (1) R_3 is CH_2 or CH_2-CH_2, Z_1 is the identifying group of Leu or Phe, or (2) R_3 is CHOH-CH_2, Z_1 is the identifying group of Leu, cyclohexyl-Ala, or Phe and each R_5 and R_6 is H; provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, Z_1 can only be F_5-Phe; and where V is NHR_6, R_6 is NH_2; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl.

Preferably, the therapeutic peptide is of the forumla wherein V is OR_4, and R_4 is any of C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl, and A^6 is N-methyl-D-Ala or A^1 is D-F_5-Phe.

The therapeutic peptide may also comprise between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring

peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

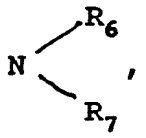
A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;
wherein R_4 is CH_2-NH, CH_2-S, CH_2-O, CO-CH_2, CH_2-CO, or CH_2-CH_2, and each Z_1 and Z_2, independently, can be the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, β-Nal, p-X-Phe (where X = H, F, Cl, Br, NO_2, OH or CH_3), Trp, Cys, Met, Pro, HyPro, or cylcohexyl-Ala; provided that, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 and Z_2 can be any one said identifying group; and provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, R^4 must be CH_2-O, and Z_1 and Z_2, independently, can be any one said identifying group; and V is either OR_5 or



where each R_3, R_5, R_6, and R_7, independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

In preferred embodiments, the therapeutic peptide is of the formula

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

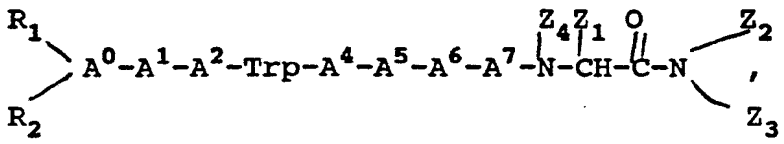
A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;

A^7 = His;

and, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, and R_4 is CH_2-NH or CH_2-O, each Z_1 and Z_2, independently, is the identifying group of Leu or Phe; provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, R_4 can only be CH_2-O, and Z_1 and Z_2, independently, can be any said identifying group; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl.

Preferably, the analog is of the formula wherein R_4 is CH_2-NH, and said carbon atom is bonded to Z_2 is of said R configuration.

The therapeutic peptide may also comprise between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of

any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

provided that, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 is the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, β-Nal, Gln, p-X-Phe (where X = H, F, Cl, Br, NO_2, OH or CH_3), F_5-Phe, Trp, Cys, Met, Pro, or HyPro; and provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, A^1 must be the identifying group of F_5-D-Phe; and each Z_2, Z_3, and Z_4, independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; further provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must

be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

In preferred embodiments, the therapeutic peptide is of the formula

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

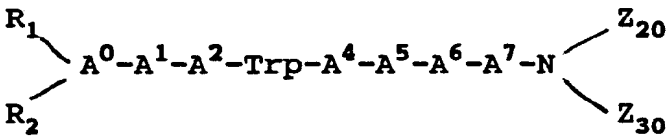
A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;

A^7 = His;

and, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 is the identifying group of any one of the amino acids Leu, F_5-Phe, or p-X-Phe (where X = H, F, Cl, Br, NO_2, OH or CH_3); provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, Z_1 can only be the identifying group of F_5-Phe; and each Z_2, Z_3 and Z_4, independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl.

The therapeutic peptide may also comprise between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met

residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H, or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

wherein each Z_{20} and Z_{30}, independently, is H, lower alkyl, lower phenylalkyl, lower naphthylalkyl; further provided

that, when either of Z_{20} or Z_{30} is other than H, A^7 is His, A^6 is Gly, A^5 is Val, A^4 is Ala, A^2 is His, and either of R_1 or R_2 is other than H, A^1 is F_5-D-Phe; further provided that either A^1 must be F_5-D-Phe or A^6 must be N-methyl-D-Ala; provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

In preferred embodiments, the therapeutic peptide is of the formula

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;

A^7 = His;

and, where each Z_{20} and Z_{30}, is H; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl; provided that either A^1 must be F_5-D-Phe or A^6 must be N-methyl-D-Ala.

In other preferred embodiments, the analog is at least 25% homologous, preferably at least 50% homologous,

with the naturally occurring peptide.

Preferred peptides of the invention include p-Glu-Gln-Trp-Ala-Val-Gly-His-statine-amide; D-Cpa-Gln-Trp-Ala-Val-Gly-His-β-Leu-NH_2; D-Cpa-Gln-Trp-Ala-Val-D-Ala-His-β-Leu-NH_2; D-Phe-Gln-Trp-Ala-Val-N-methyl-D-Ala-His-Leu-methylester; and D-F_5-Phe-Gln-Trp-Ala-Val-D-Ala-His-Leu-methylester.

Examples of additional preferred bombesin or GRP peptides are: D-β-Nal-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Phe-NH_2, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-ethylamide, p-Glu-Gln-Trp-Ala-Val-Gly-His-statine-amide, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]-D-Phe-NH_2,D-Cpa-Gln-Trp-Ala-Val-Gly-His-β-Leu-NH_2, D-Cpa-Gln-Trp-Ala-Val-D-Ala-His-β-Leu-NH_2, D-Cpa-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]-Phe-NH_2.

Examples of preferred peptides are D-Phe-Gln-Trp-Ala-Val-N-methyl-D-Ala-His-Leu-methylester. D-F_5-Phe-Gln-Trp-Ala-Val-D-Ala-His-Leu-methylester.

(Non-peptide bonds in which the peptide bond is reduced are symbolized herein by "ψ[CH_2NH]" or "ψ".)

Antagonists of the invention are useful for treating diseases involving the malignant or benign proliferation of tissue, such as all forms of cancer where bombesin-related or GRP-related substances act as autocrine or paracrine mitotic factors, e.g., cancers of the gastrointestinal tract, pancreatic cancer, colon cancer, lung cancer, particularly the small cell subtype, prostate or breast cancer; or for treating artherosclerosis, and disorders of gastrointestinal tissues related to gastric and pancreatic secretions and motility; for example, for causing the suppression of amylase secretion, or for appetite control.

In the generic formulas given above, any R or Z

group is an aromatic, lipophilic group, the in vivo activity can be long lasting, and delivery of the compounds of the invention to the target tissue can be facilitated.

The identifying group of an α-amino acid is the atom or group of atoms, other than the α-carbonyl carbon atom, the α-amino nitrogen atom, or the H atom, bound to the asymmetric α-carbon atom. To illustrate by examples, the identifying group of alanine is CH_3, the identifying group of valine is $(CH_3)_2CH$, the identifying group of lysine is $H_3N^+(CH_2)_4$ and the identifying group of phenylalanine is $(C_6H_6)CH_2$. The identifying group of a β- or γ-amino acid is the analagous atom or group of atoms bound to respectively, the β- or the γ-carbon atom. Where the identifying group of an amino acid is not specified it may be α, β, or γ.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We first briefly describe the drawings.

Drawings

Fig. 1 is a graph of tumor growth curves for the small cell lung cancer (NCI-H69) xenografts.

Fig. 2 is a series of amino acid sequences of naturally occurring peptides of which peptides of the invention are analogs.

Structure

Peptides of the invention have either a non-peptide bond in at least one of the indicated positions, or a statine, β-amino acid, or Y-amino acid substitution, e.g., sta^8-des-Met^9 litorin. By non-peptide bond is meant e.g., that the carbon atom participating in the bond between two residues is reduced from a carbonyl carbon to a methylene carbon. The peptide bond reduction

method which yields this non-peptide bond is described in Coy et al., U.S. patent application, Serial No. 879,348, assigned to the same assignee as the present application, hereby incorporated by reference. Any one of the amino acids in positions 0, 1, 2, and 9 of the litorin antagonists may be deleted from the peptides, and the peptides are still active as antagonists.

The peptides of the invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, salicylic, methanesulfonic, toluenesulfonic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric acid.

Synthesis of Litorin and Bombesin Analogs

The synthesis of the bombesin antagonist pGlu-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 follows. Other bombesin or GRP antagonists can be prepared by making appropriate modifications of the following synthetic methods.

The first step is the preparation of the intermediate pGlu-Gln-Trp-Ala-Val-Gly-His(benzyloxycarbonyl)-Leuψ[CH_2NH]Leu-benzhydrylamine resin, as follows.

Benzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) (0.97 g, 0.5 mmole) in the chloride ion form is placed in the reaction vessel of a Beckman 990B peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid (TFA) in methylene chloride (2 times for 1 and 25 min. each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; and (f) 10% triethylamine

in chloroform.

The neutralized resin is stirred with alpha-t-butoxycarbonyl(Boc)-leucine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hour, and the resulting amino acid resin is then cycled through steps (a) to (f) in the above wash program. Boc-leucine aldehyde (1.25 mmoles), prepared by the method of Fehrentz and Castro, Synthesis, p. 676 (1983), is dissolved in 5 ml of dry dimethylformamide (DMF) and added to the resin TFA salt suspension followed by the addition of 100 mg (2 mmoles) of sodium cyanoborohydride (Sasaki and Coy, Peptides 8:119-121 (1987); Coy et al., id.). After stirring for 1 hour, the resin mixture is found to be negative to ninhydrin reaction (1 min.), indicating complete derivatization of the free amino group.

The following amino acids (1.5 mmole) are then coupled successively in the presence diisopropylcarbodiimide (1.5 mmole), and the resulting amino acid resin is cycled through washing/deblocking steps (a) to (f) in the same procedure as above: Boc-His(benzyloxycarbonyl), Boc-Gly (coupled as a 6 M excess of the p-nitrophenylester), Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled as a 6 M excess of the p-nitrophenylester), and pGlu. The completed resin is then washed with methanol and air dried.

The resin described above (1.6 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen, and free peptide is precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column (2.5 x 100 mm) of Sephadex G-25 (Pharmacia Fine Chemicals, Inc.). Fractions containing a major component by uv absorption and thin layer chromatography (TLC) are then pooled, evaporated to a

small volume and applied to a column (2.5 x 50 cm) of octadecylsilane-silica (Whatman LRP-1, 15-20 μm mesh size).

The peptide is eluted with a linear gradient of 0-30% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by TLC and analytical high performance liquid chromatography (HPLC) and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 60 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by HPLC and TLC. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide. The presence of the Leuψ[CH_2-NH]Leu bond is demonstrated by fast atom bombardment mass spectrometry.

pGlu-Gln-Trp-Ala-Val-Gly-His-Pheψ[CH_2NH]Leu-NH_2 and pGlu-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 or other peptides are prepared in similar yields in an analogous fashion by appropriately modifying the above procedure.

Solid phase synthesis of D-Phe^1, Leu$^8\psi$[CH_2NH]D-Phe^9-litorin, D-Phe-Gln-Trp-Ala- Val- Gly-His-Leuψ[CH_2NH]-D-Phe-NH_2, was carried out as follows: Boc-D-Phe-Gln-Trp-Ala-Val-Gly-His(tosyl)-Leuψ[CH_2NH]-D-Phe-benzhydrylamine resin was synthesized first.

Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc.) (1.25 g, 0.5 mmole) in the chloride ion form is placed in the reaction vessel of an Advanced ChemTech ACT 200 peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 25 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform.

The neutralized resin is stirred with Boc-D-phenylalanine and diisopropylcarbodiimide (1.5 mmole

each) in methylene chloride for 1 h and the resulting amino acid resin is then cycled through steps (a) to (g) in the above wash program. The Boc group is then removed by TFA treatment. Boc-leucine aldehyde (1.25 mmoles), prepared by the method of Fehrentz and Castro (1), is dissolved in 5 ml of dry DMF and added to the resin TFA salt suspension followed by the addition of 100 mg (2 mmoles) of sodium cyanoborohydride (2,3). After stirring for 1 h, the resin mixure is found to be negative to ninhydrin reaction (1 min) indicating complete derivatization of the free amino group.

The following amino acids (1.5 mmole) are then coupled successively by the same procedure: Boc-His(benzyloxycarbonyl), Boc-Gly, Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled in the presence of 1 equiv. hydroxybenzotriazole), Boc-D-Phe (coupled in the presence of 1 equiv. hydroxybenzotriazole). After drying, the peptide resin weighed 1.93 g.

The resin (1.93 g, 0.5 mmole) is mixed with anisole (5 ml) and anhydrous hydrogen fluoride (35 ml) at 0°C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column (2.5 x 100 mm) of Sephadex G-25. Fractions containing a major component by uv absorption and thin layer chromatography are then pooled, evaporated to a small volume and applied to a column (2.5 x 50 cm) of Vydac octadecylsilane (10-15 uM). This is eluted with a linear gradient of 15-45% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophlization of the solution from water gives 120 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the octapeptide. The presence of the Leuψ[CH_2NH] peptide bond is demonstrated by fast atom bombardment mass spectrometry.

Solid phase synthesis of D-Phe^1-Leu^{8}des-Met^9 litorin, D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2, was carried out as follows.

Step (1): Benzhydrylamine-polystyrene resin (Advanced ChemTech, Inc. (0.62 gm, 0.25 mmole) in the chloride ion form is placed in the reacton vessel of an ACT 200 peptide synthesizer programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 25 min each); (c) methylene chloride; (d) ethanol; (e) methylene chloride; (f) 10% triethylamine in chloroform.

The neutralized resin is stirred with Boc-leucine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 hr and the resulting amino acid resin is then cycled through steps (a) to (g) in the above wash program. The following amino acids (1.5 mmole) are then coupled successively by the same procedure: Boc-His (benzyloxycarbonyl), Boc-Gly, Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled as a 6M excess of the p-nitrophenylester, and pGlu (coupled in the presence of hydroxzybenzotriazole). After drying, the peptide resin weighed 0.92 g.

Step (2): The resin (0.92 g) is then mixed with anisole (5 ml), dithiothreitol (200 mg) and anhydrous hydrogen fluoride (35 ml) at 0° C and stirred for 45 min. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen and free peptide precipitated and washed with ether. The crude peptide is dissolved in a minimum volume of 2 M acetic acid and eluted on a column

(2.5 x 100 cm) of Sephadex G-25. Fractions containing a major component by UV absorption and thin layer chromatography are then pooled, evaporated to a small volume and applied to a column (2.5 x 50 cm) of Vydac octadecylsilane (10-15 microM). The column is eluted with a linear gradient of 0-30% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gives a white, fluffy powder; this product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the peptide.

Synthesis of D-Nal-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2 was accomplished using the same procedure as described above (0.62 g, 0.25 mmole of benzyhydrylamine resin in step (1), and 0.92 g in step (2)).

Synthesis of N-acetyl-D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2 was accomplished using the same procedure as that described above, using 0.62 g (0.25 mmole) of benzhydrylamine resin in step (1), and mixing 0.92 g of the resin with anisole in step(2), except that the final Boc group was removed and the resin acetylated with acetic anhydride in methylene chloride.

Synthesis of D-Phe-Gln-Trp-Ala-Val-N-Me-D-Ala-His(Tos)-Leu-O-resin is as follows:

Boc-Leu-O-Merrifield resin (1.0 g. 0.5 mmole) is placed in the reaction vessel of an Advanced ChemTech ACT 200 automatic peptide synthesizer programmed to perform the following reaction/wash cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid in methylene chloride (2 times for 1 and 25 min. each); (c) propanol; (d) dimethylformamide; (e) 10% triethylamine in dimethylformamide; (f) dimethylformamide.

The neutralized resin is stirred with Boc-N^{im}-tosyl-histidine and diisopropylcarbodiimide (1.5 mmole each) in methylene chloride for 1 h. and the resulting amino acid resin is then cycled through steps (a) to (f) in the above wash program. The Boc group is then removed by TFA treatment. The following amino acids (1.5 mmole) are then coupled successively by the same procedure: Boc-N-Me-D-Ala (purchased from Bachem, Inc., CA), Boc-Val, Boc-Ala, Boc-Trp, Boc-Gln (coupled in the presence of 1 equiv. hydroxybenzotriazole), and Boc-D-Phe. After the last coupling was complete, the final Boc group was removed by TFA treatment as already described. After drying, the peptide resin weighed 1.28 g.

Synthesis of D-F_5-Phe-Gln-Trp-Ala-Val-D-Ala-His(Tos)-Leu-O-resin is as follows:

This analogue is prepared under the same conditions described above, except that Boc-D-Ala is used in place of N-Me-D-Ala and Boc-D-F_5Phe in place of D-Phe.

Synthesis of D-F_5Phe-Gln-Trp-Ala-Val-D-Ala-His-Leu-methyl ester is as follows.

This peptide is cleaved from the Merrifield resin described above under the same conditions, to give 198 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the octapeptide and fast atom bombardment mass spectrometry gives the expected molecular weight for the peptide.

Synthesis of D-Phe-Gln-Trp-Ala-Val-N-Me-D-Ala-His-Leu-methyl ester is as follows:

The Merrifield resin described above (1.28 g, 0.5

mmole) is suspended in methanol containing 10% triethylamine and stirred at room temperature for 3 days. After filtration, the solution is evaporated to an oil which is dissolved in water and eluted on a column of Vydac octadecylsilane (10-15 μM) with a linear gradient of 10-40% acetonitrile in 0.1% trifluoroacetic acid in water. Fractions are examined by thin layer chromatography and analytical high performance liquid chromatography and pooled to give maximum purity. Repeated lyophilization of the solution from water gives 49 mg of the product as a white, fluffy powder.

The product is found to be homogeneous by hplc and tlc. Amino acid analysis of an acid hydrolysate confirms the composition of the octapeptide and fast atom bombardment mass spectrometry gives the expected molecular weight for the peptide.

The synthesis of Sta^8-des-Met^9 litorin follows. A statine, AHPPA, or ACHPA residue can be substituted in place of any two amino acids of the peptide, where the peptide contains only peptide bonds. For example, sta^8-des Met^9 litorin was prepared in an analagous fashion by first coupling statine to the resin and then proceeding with the addition of Boc-His(benzylocarbonyl). Statine or Boc-statine can be synthesized according to the method of Rich et al., 1978, J. Organic Chem. 43; 3624; and Rich et al., 1980, J. Med. Chem. 23: 27, and AHPPA and ACHPA can be synthesized according to the method of Hui et al., 1987, J. Med. Chem. 30: 1287; Schuda et al., 1988, J. Org. Chem. 53:873; and Rich et al., 1988, J. Org. Chem. 53:869.

Solid-phase synthesis of the peptide BIM-26120, pGlu-Gln-Trp-Ala-Val-Gly-His-Sta-NH_2, was accomplished through the use of the following procedures in which alpha-t-butoxycarbonyl statine (prepared by the procedure of Rich et al., J. Org. Chem. 1978, 43, 3624) is first

coupled to methylbenzhydrylamine-polystyrene resin. After acetylation, the intermediate p-Glu-Gln-Gln-Trp-Ala-Val-Gly-His(benzyloxycarbony1)-Sta-methylbenzhydrylamine resin is prepared. The synthetic procedure used for this preparation follows in detail:

1. Incorporation of alpha-t-butoxycarbonyl statine on methylbenzhydrylamine resin.

Methylbenzhydrylamine-polystyrene resin (Vega Biochemicals, Inc.) (1.0 g, 0.73 mmol) in the chloride ion form is placed in the reaction vessel of a Vega 250C Coupler peptide synthesizer. The synthesizer was programmed to perform the following reactions: (a) methylene chloride; (b) 10% triethylamine in chloroform; (c) methylene chloride; and (d) dimethylformamide.

The neutralized resin is mixed for 18 hours with the preformed active ester made from alpha-t-butoxycarbonyl statine (1.46 mmol), diisopropyl carbodiimide (2 mmol), and hydroxybenzotriazole hydrate (1.46 mmol in dimethylformamide at 0° C. for one hour. The resulting amino acid resin is washed on the synthesizer with dimethylformamide and then methylene chloride. The resin mixture at this point was found by the Kaiser ninhydrin test (5 minutes) to have an 84% level of statine incorporation on the resin.

Acetylation was performed by mixing the amino-acid resin for 15 minutes with N-acetyl imidazole (5 mmol) in methylene chloride. Derivatization to the 94-99% level of the free amino groups of the resin was indicated by the Kaiser ninhydrin test (5 minutes). The Boc-statine-resin is then washed with methylene chloride.

2. Couplings of the Remaining Amino Acids.

The peptide synthesizer is programmed to perform the following reaction cycle: (a) methylene chloride; (b) 33% trifluoroacetic acid (TFA) in methylene chloride (2 times for 5 and 25 min. each); (c) methylene chloride; (d)

isopropyl alcohol; (e) 10% triethylamine in chloroform; and (f) methylene chloride.

The following amino acids (2.19 mmol) are then coupled successively by diisopropyl carbodiimide (4 mmol) alone or diisopropyl carbodiimide (4 mmol) plus hydroxybenzotriazole hydrate (1.47 or 0.73 mmol) and the resulting peptide-resin is washed on the synthesizer with dimethylformamide and then methylene chloride, and then cycled through the washing and deblocking steps (a) to (f) in the procedure described above.

Boc-His (benzyloxycarbonyl) (coupled in the presence of 2 equivalents hydroxybenzotriazole); Boc-Gly; Boc-Val; Boc-Ala and Boc-Trp (coupled as the preformed hydroxybenzotriazole active esters made by reaction at 0° C for one hour with 1 equivalent hydroxybenzotriazole hydrate); Boc-Gln and pGlu (also coupled as the preformed active esters of hydroxybenzotriazole made by reaction at 0° C for one hour with 1 equivalent hydroxybenzotriazole hydrate). The completed peptide-resin is then washed with methanol and air dried.

The peptide-resin described above (1.60 g, 0.73 mmol) is mixed with anisole (2.5 mL), dithioerythreitol (50 mg), and anhydrous hydrogen fluoride (30 mL) at 0° C. for one hour. Excess hydrogen fluoride is evaporated rapidly under a stream of dry nitrogen, and the free peptide is precipitated and washed with ether. The crude peptide is dissolved in 100 mL of 1 M acetic acid and the solution is then evaporated under reduced pressure. The crude peptide is dissolved in a minimum volume of methanol/water 1/1 and triturated with 10 volumes of ethyl acetate.

The triturated peptide is applied to a column (9.4 mm I.D. x 50 cm) of octadecylsilane-silica (Whatman Partisil 10 ODS-2 M 9). The peptide is eluted with a linear gradient of 20-80% of 20/80 0.1% trifluoroacetic acid/acetonitrile in 0.1% trifluoroacetic acid in water.

Fractions are examined by TLC and analytical high performance liquid chromatography (HPLC) and pooled to give maximum purity. Lyophilization of the solution from water gives 77 mg of the product as a white fluffy powder.

Other compounds including D-Cpa^1, β-leu^8, desMet^9 Litorin can be prepared as above and tested for effectiveness as agonists or antagonists in the test program described below.

A statine, AHPPA, ACHPA, β-amino acid, or Y-amino acid residue is added in the same way as is a natural α-amino acid residue, by coupling as a Boc derivative.

Phase 1 - 3T3 Peptide Stimulated [^{3}H] Thymidine Uptake Assay

Cell Culture. Stock cultures of Swiss 3T3 cells are grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum in humidified atmosphere of 10% CO_2/90% air at 37°C. For experimental use, the cells are seeded into 24-well cluster trays and used four days after the last change of medium. The cells are arrested in the G1/G0 phase of the cell cycle by changing to serum-free DMEM 24 hours prior to the thymidine uptake assay.

Assay of DNA Synthesis. The cells are washed twice with 1ml aliquots of DMEM (-serum) then incubated with DMEM (-serum), 0.5μM [methyl-^{3}H] thymidine (20Ci/mmole, New England Nuclear), bombesin (3nM), and initially four concentrations of the test compounds (1, 10, 100, 1000nM) in a final volume of 1.0 ml. After 28 hours at 37°C, [methyl-^{3}H] thymidine incorporation into acid-insoluble pools is assayed as follows. The cells are washed twice with ice-cold 0.9% NaCl (1ml aliquots), and acid soluble radioactivity is removed by a 30 min. (4°C) incubation with 5% trichloroacetic acid (TCA). The cultures are then washed once (1ml) with 95% ethanol and

solubilized by a 30 min. incubation (1ml) with 0.1N NaOH. The solubilized material is transferred to vials containing 10ml ScintA (Packard), and the radioactivity is determined by liquid scintillation spectrometry.

Phase 2 - Small Cell Carcinoma (SCLC) - Bombesin Stimulated [^{3}H] Thymidine Uptake Assay

Cell Culture. Cultures of the human cell carcinoma cell line (NCI-H69) (obtained from the American Type Culture Association) are maintained in RPMI 1640 medium supplemented with 10% fetal calf serum in 10% CO_2/90% air at 37°C. Twenty-four hours prior to assay, the cells are washed with serum-free medium and seeded in 24-well cluster trays.

Assay of DNA Synthesis. Bombesin (1nM), 0.5μM [methyl-^{3}H] thymidine (20 Ci/mmole, New England Nuclear), and four concentrations of the test compounds (1, 10, 100, 1000nM) are added to the cultures to achieve a final volume of 0.5 ml. After a 28 hr incubation at 37°C, the cells are collected onto GF/B glass fiber filters, and the DNA is precipitated with ice-cold TCA. [^{3}H] thymidine incorporation into acid-insoluble fractions of DNA is determined by liquid scintillation spectrometry.

Phase 3 - Peptide-Induced Pancreatitis

Male, Sprague-Dawley rats (250g) are used for these experiments. The test compound, or 0.9% NaCl is administered s.c. 15 min. prior to the bombesin injection. Bombesin injections are given s.c. at a dose of 10 μg/kg, and blood samples are obtained at 1 hr.30 min., 3hr. and 6hr. Plasma amylase concentration are determined by the Pantrak Amylase test.

Phase 4- In Vitro Inhibition of [^{125}I] Gastrin Releasing Peptide (GRP) Binding to Bombesin Receptors

Membranes from various tissues (rat brain, rat pancreas, rat anterior pituitary, SCLC, 3T3 cells) are

prepared by homogenization in 50mM TrisHCl containing 0.1% bovine serum albumin and 0.1mg/ml bacitracin followed by two centrifugations (39,000xgx15 min., 4°C) with an intermediate resuspension in fresh buffer. For assay, aliquots (0.8ml) are incubated with 0.5nM [^{125}I]GRP (~2000 Ci/mmol, Amersham Corp.) and various concentrations of the test compounds in a final volume of 0.5ml. After a 30 minute incubation at 4°C, the binding reaction is terminated by rapid filtration through Whatman GF/C filters that have been pre-soaked in 0.3% aqueous polethyleneimine to reduce the level of nonspecific binding. The filters and tubes are washed three times with 4ml aliquots of ice-cold buffer, and the radioactivity trapped on the filters is counted by gamma-spectrometry. Specific binding is defined as the total [^{125}I]GRP bound minus that bound in the presence of 1000nM bombesin or a related peptide.

Phase 5- Inhibition of Gastrin Release

The stomachs of anesthetized rats are perfused with saline collected over 15 minute periods via pyloric cannulation while the test peptide is infused through the femoral vein for periods between 0 and 150 minutes.

Phase 6- In Vivo Antitumor Activity

NCI-H69 small cell lung carcinoma cells were transplanted from in vitro culture by implanting each animal with the equivalent of 5 confluent 75 cm^2 tissue culture flasks in the right flank. In vitro NCI-H69 cells grow as a suspension of cellular aggregates. Therefore, no attempt was made to disaggregate the cell agglomerates by physical or chemical means. Tumor size was calculated as the average of two diameters, i.e., (length and width/2) mm.

Results of Assays of Test Peptides

A number of analogs of bombesin or GRP, each containing a non-peptide bond or a statine, AHPPA, or

ACHPA, β-amino acid, or Y-amino acid residue, can be synthesized and tested in one or more of the above-described Phase 1 - 6 assays; the results of Phase 1 and 2 tests are given in Table 1 attached hereto. Table 1 shows formulas for the non-peptide analogues and results of in vitro inhibition of [^{125}I]GRP binding to 3T3 fibroblast bombesin receptors, and bombesin-stimulated [^{3}H]Thymidine uptake by cultured 3T3 cells. (3T3 GRP receptor and thymidine uptake data are expressed in IC50 (nM).) Table 1 also gives results for non-peptide bond-containing analogs of one other naturally-occurring peptide, Neuromedin C, whose C-terminal seven amino acids are similar to those of bombesin and GRP. (In Table 1, "Litorin" indicates a 9 residue peptide analog or its derivative, whereas "Neuromedin C" indicates a 10 residue analog or its derivative.)

In Table 1, the position of the non-peptide bond is indicated by the position of the symbol $\psi[CH_2NH]$; i.e., $\psi[CH_2NH]$ is always shown preceding the amino acid which, in that peptide, is bonded to the amino acid N-terminal to it via the non-peptide bond. Where no amino acid is specified under "structure", the non-peptide bond links the two peptides represented by the numbers given as post-scripts.

In Table 1, it can be seen that a preferred placement of the non-peptide bond in litorin analogs is at the A^8 - A^9 position; two of the most active analogs (as indicated by a low GRP receptor IC50 value) are BIM-26100 ($Phe^8\psi[CH_2NH]Leu^9$) and BIM-26101 ($Leu^8\psi[CH_2NH]Leu^9$).

In addition, as shown in Table 1, BIM-26113 (D-Phe^1, $Leu^8\psi[CH_2NH]Leu^9$) and BIM-26114 (D-Nal^1, $Leu^8\psi[CH_2NH]Leu^9$) are active in the 3T3 GRP receptor binding and thymidine uptake assays. Most notably, BIM-26136 (D-Nal^1, $Leu^8\psi[CH_2NH]Phe^9$), which contains amino

and carboxy terminal aromatic residues that are capable of forming a hydrophobic interaction, is the most potent analog. Finally, when statine or β-leucine replaces the A^8 and A^9 residues of litorin, the resultant analogs BIM-26120 and BIM-26182 are also potent antagonists.

Table 1 also shows that Neuromedin C analogs containing a non-peptide bond between residues A^9 - A^{10}, e.g., BIM-26092, 26105, 26106, and 26107, are antagonists when tested in the 3T3 GRP receptor and thymidine uptake assays.

Table 1 also gives negative results for analogs of Neuromedin C and GRP 19-27, e.g., BIM-26108. Thus the non-peptide bond placement guidelines given herein should be used in conjunction with the routine assays described above to select useful antagonists.

Bombesin and Bombesin analogs have been shown to inhibit the effect of interleukin-2 (IL-2) (Fink et al., 1988, Klin. Wochenschr. 66, Suppl. 13, 273). Since IL-2 causes T lymphocytes to proliferate, it is possible that litorin antagonists may prevent the inhibitory effect of Bombesin or its analogs on IL-2. IL-2 stimulated lymphocytes are capable of effectively lysing small cell lung carcinoma cells in vitro. Although Bombesin antagonists have a direct antiproliferative effect on neoplastic tissues, they may also favor proliferation of lymphocytes having lytic activity for small cell lung carcinoma.

These observations prompted us to evaluate the effect of BIM-26100 on the in vivo growth of the SCLC tumor cell line described in Phase 6. Twenty athymic nude females, 5 to 6 weeks of age, were implanted on day 0 with the NCI-H69 human SCLC, individually identified and then randomized into the following vehicle control and test groups:

Group No.	Treatment	No. Animals
1	Saline vehicle treated control: 0.2 ml, s.c. inf., b.i.d., QD1-28	10
2	BIM-26100: 50ug/inj., s.c., b.i.d., QD1-28	5
3	BIM-26100: 50ug/inj., s.c. inf., b.i.d., QD1-28	5

(s.c. = subcutaneously; inf. = infused around tumor; inj. = injected; b.i.d. = twice per day; QD1-28 = daily treatment, on days 1 - 28.)

Growth of NCI-H69 xenografts and the tumor growth inhibitory activity of the bombesin antagonist BIM-26100 (pGlu-Gln-Trp-Ala-Val-Gly-His-Pheψ[CH_2NH]Leu-NH_2) are illustrated as tumor growth curves in Fig. 1, and relative tumor sizes in Table 2. Administration of BIM-26100 as a s.c. infusion around the tumor significantly inhibited tumor growth. The effectiveness of the antitumor activity of BIM-26100 is evident in view of the large inoculum of NCI-H69 tumor cells (i.e., the equivalent of 5 confluent 75 cm^2 cell culture flasks per animal) and the agglomerated condition of the cells. In confluent flasks, NCI-H69 agglomerates are macroscopically visible and together resemble a metastatic tumor colony. Many such tumor colonies were implanted per animal. The dose of BIM-26100 was arbitrarily selected on the basis of compound availability and is not optimal. Higher doses of BIM-26100 may be administered, as indicated by body weight gain (minus tumor weight) gain during the course of treatment (Table 3). This suggests BIM-26100 completely lacks local or systemic toxicity and is useful therapeutically as an anti-growth factor with anti-tumor effects.

D-Phe-Gln-Trp-Ala-Val-N-methyl-D-Ala-His-Leu-methylester and D-F_5-Phe-Gln-Trp-Ala-Val-D-Ala-His-Leu-methylester were examined for their abilities to displace ^{125}I-labeled bombesin from rat pancreatic acini cells and to inhibit amylase release from these cells produced by

bombesin itself. The analogues exhibit potencies in the half-maximal effective dose ranges of 5-10 nM and are thus potent bombesin receptor antagonists.

Use

The peptides of the invention may be administered to a mammal, particularly a human, in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), in a sustained release formulation using a biodegradable biocompatible polymer, or by on-site delivery (e.g., in the case of anti-cancer bombesin to the lungs) using micelles, gels and liposomes.

The bombesin antagonists of the invention are suitable for the treatment of all forms of cancer where bombesin-related substances act as autocrine or paracrine mitotic agents, particularly small-cell lung carcinoma. The peptides can also be used for the inhibition of gastric acid secretion and motility disorders of the GI tract, the symptomatic relief and/or treatment of exocrine pancreatic adenocarcinoma, and the restoration of appetite to cachexic patients. The peptides can be administered to a human patient in a dosage of 0.5 μg/kg/day to 5 mg/kg/day. For some forms of cancer, e.g., small cell lung carcinoma, the preferred dosage for curative treatment is 250mg/patient/day.

The compound can be administered to a mammal, e.g., a human, in the dosages used for growth hormone releasing factor or, because of their decreased potency, in larger dosages. The compounds can be administred to a mammal, e.g., a human, in a dosage of 0.01 to 1000 mcg/kg/day, preferably 0.1 to 100 mcg/kg/day.

Table 1

Code	Structure	3T3 GRP Receptor IC50(nM)	Thym. Uptake IC50(nM)
BIM-26092	Gly-Asn-His-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 Neuromedin C	242	466
BIM-26095	pGlu-Gln-Trp-Ala-Val-D-Ala-His-Leuψ[CH_2NH]Leu-NH_2 Litorin	2623	1209
BIM-26100	pGlu-Gln-Trp-Ala-Val-Gly-His-Pheψ[CH_2NH]Leu-NH_2 Litorin	23	26
BIM-26101	pGlu-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 Litorin	118	296
BIM-26105	D-Ala-Asn-His-Trp-Ala-Val-D-ALa-His-Leuψ[CH_2CH]Leu-NH_2 Neuromedin C	107	107
BIM-26106	desGly-D-Ala-His-Trp-Ala-Val-D-Ala-His-Leuψ[CH_2NH]Met-NH_2 Neuromedin C	401	354
BIM-26107	D-Phe-His-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 Neuromedin C	199	154

Table 1 (cont'd)

Code	Structure	3T3 GRP Receptor IC50(nM)	Thym. Uptake IC50(nM)
BIM-26108	N-Ac-D-Ala-His-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 GRP (19-27)	841	>1000
BIM-26113	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 Litorin	5.8	9
BIM-26114	D-Nal-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Leu-NH_2 Litorin	23.5	28
BIM-26120	pGlu-Gln-Trp-Ala-Val-Gly-His-Sta-NH_2 Litorin	150	165
BIM-26122	D-Phe-Gln-Trp-Ala-Val-Gly-His-Leu-NH_2 Litorin	5.9	28.6
BIM-26136	D-Nal-Gln-Trp-Ala-Val-Gly-His-Leuψ[CH_2NH]Phe-NH_2 Litorin	1.4	3.3
BIM-26182	D-Cpa-Gln-Trp-Ala-Val-Gly-His-β-Leu-NH_2 Litorin	0.88	4.77

TABLE II

IN VIVO TUMOR INHIBITORY ACTIVITY OF THE BOMBESIN ANTAGONIST BIM-21600: NCI-H69 HUMAN SCLC

Group No.	Treatment	Tumor Size[1] Day 18 (mm)	% Test/ Control	Tumor Size Day 28 (mm)	% Test/ Control
1	Vehicle treated control, 0.2 ml, s.c. inf., b.i.d., QD1-28	10.9±1.82		15.9±2.27	
2	BIM-26100, 50 µg/inj., s.c., b.i.d., QD1-28	10.1±1.47	93	17.3±1.96	108
3	BIM-26100, 50 µg/inj., s.c. inf., b.i.d., QD1-28	7.6±1.56**	70	13.7±0.67*	86

[1] Data reported as means ± SD on 10 animals in the control and 5 in test groups.

Student's t Test significance of difference from control: *p<0.05; **p<0.01

TABLE III

EFFECT OF TUMOR GROWTH AND BIM-26100 TREATMENT ON BODY WEIGHT: LACK OF SYSTEMIC TOXICITY

Group No.	Treatment	Body Weight (gm)[1] Day 0	Body Weight (gm) Day 18	Body Weight (gm) Day 28
1	Vehicle treated control, 0.2 ml, s.c. inf., b.i.d., QD1-28	17.3	19.6	19.7
2	BIM-26100, 50 μg/inj., s.c., b.i.d., QD1-28	16.9	19.2	19.1
3	BIM-26100, 50 μg/inj., s.c. inf., b.i.d., QD1-28	17.7	20.4	21.1

[1] Body weights are presented as means of 10 animals in the control and 5 in test groups.
Tumor weights calculated from 2 largest diameters in mm converted to mgs using the formula for an ellipsoid (length x width ^{2}/2) mgs, were subtracted from total body weights.

Other embodiments are within the following claims.

Claims

1. A linear peptide which is an analog of naturally occurring, biologically active bombesin, or mammalian gastrin releasing peptide, having an active site and a binding site responsible for the binding of said peptide to a receptor on a target cell, cleavage of a peptide bond in said active site of naturally occurring bombesin, or mammalian gastrin releasing peptide being unnecessary for in vivo biological activity, said analog having one of the following modifications: (1) a replacement of two amino acid residues within said active site with a β-amino acid, or a γ-amino acid residue, (2) a non-peptide bond instead of a peptide bond between an amino acid residue of said active site and an adjacent amino acid residue, (3) a deletion of one carboxy-terminal amino acid of said peptide which is accompanied by the carboxy-terminal amino acid being of the ester or amide form, or (4) alkylation of the amide bond between the penultimate and the ultimate carboxy-terminal amino acid.

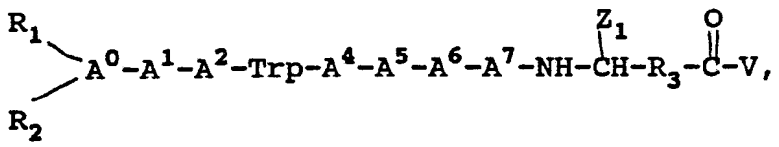
2. The linear peptide of claim 1 wherein said analog is capable of binding to said receptor, so that said analog is capable of acting as a competitive inhibitor of said naturally occurring peptide by binding to said receptor and, by virtue of one of said modifications, failing to exhibit the in vivo biological activity of naturally occurring bombesin or mammalian gastrin releasing peptide.

3. The linear peptide of claim 1 wherein said active site comprises at least one amino acid in the carboxy terminal half of the naturally occurring, biologically active peptide, said linear peptide

including said amino acid residue in its carboxy terminal half.

4. The linear peptide of claim 1 wherein said binding site comprises at least one amino acid in the amino terminal half of the naturally occurring, biologically active peptide, said linear peptide including said amino acid residue in its amino terminal half.

5. A therapeutic peptide comprising between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met,

p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

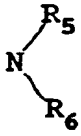
A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

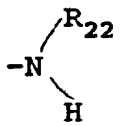
A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

wherein R_3 is $CHR_{20}-(CH_2)_{n1}$ (where R_{20} is either of H or OH; and n1 is either of 1 or 0), or is deleted, and provided that where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 is the identifying group of any of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, p-X-Phe (where X = H, F, Cl, Br, NO_2, OH, or CH_3), F_5-Phe, Trp, Cys, Met, Pro, HyPro, cyclohexyl-Ala, or β-nal; provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, Z_1 can only be F_5-Phe; and V is either

OR_4, or  ,

where R_4 is any of C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl, and each R_5, and R_6, independently, is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, lower acyl, or,

 ,

where R_{22} is any of H, C_{1-12} alkyl, C_{7-10} phenylalkyl, or lower acyl; provided that, when one of R_5 or R_6 is $-NHR_{22}$, the other is H; provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

6. The therapeutic peptide of claim 5 wherein

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

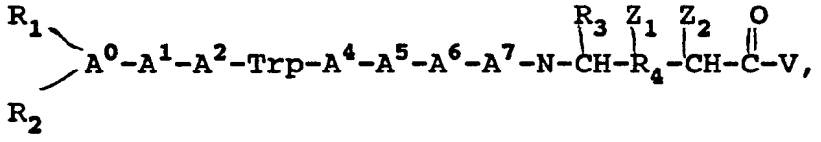
A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;

A^7 = His;

and, where A^1 is F^5-D-Phe or A^6 is N-methyl-D-Ala, (1) R_3 is CH_2 or CH_2-CH_2, Z_1 is the identifying group of Leu or Phe, or (2) R_3 is CHOH-CH_2, Z_1 is the identifying group of Leu, cyclohexyl-Ala, or Phe and each R_5 and R_6 is H; provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, Z_1 can only be F_5-Phe; and

where V is NHR_6, R_6 is NH_2; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl.

7. A therapeutic peptide comprising between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

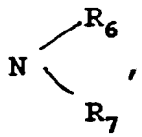
A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

wherein R_4 is CH_2-NH, CH_2-S, CH_2-O, CO-CH_2, CH_2-CO, or CH_2-CH_2, and each Z_1 and Z_2, independently, can be the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, Gln, β-Nal, p-X-Phe (where X = H, F, Cl, Br, NO_2, OH or CH_3), Trp, Cys, Met, Pro, HyPro, or cylcohexyl-Ala; provided that, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 and Z_2 can be any one said identifying group; and provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, R^4 must be CH_2-O, and Z_1 and Z_2, independently, can be any one said identifying group; and V is either OR_5 or

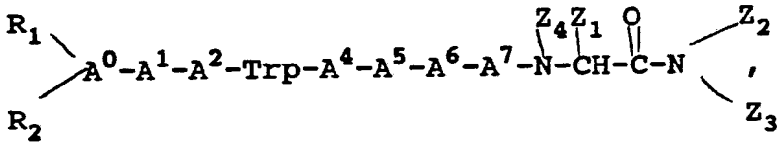


where each R_3, R_5, R_6, and R_7, independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20}

alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

8. The therapeutic peptide of claim 7 wherein
A^0 = Gly, D-Phe, or is deleted;
A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;
A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;
A^4 = Ala;
A^5 = Val;
A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;
A^7 = His;
and, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, and R_4 is CH_2-NH or CH_2-O, each Z_1 and Z_2, independently, is the identifying group of Leu or Phe; provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, R_4 can only be CH_2-O, and Z_1 and Z_2, independently, can be any said identifying group; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl.

9. A therapeutic peptide comprising between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

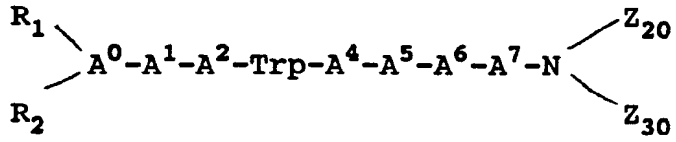
A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

provided that, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 is the identifying group of any one of the amino acids Gly, Ala, Val, Leu, Ile, Ser, Asp, Asn, Glu, β-Nal, Gln, p-X-Phe (where X = H, F, Cl, Br, NO_2, OH or CH_3), F_5-Phe, Trp, Cys, Met, Pro, or HyPro; and provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, A^1 must be the identifying group of F_5-D-Phe; and each Z_2, Z_3, and Z_4, independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; further provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; and further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

10. The therapeutic peptide of claim 9 wherein
A^0 = Gly, D-Phe, or is deleted;
A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;
A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;
A^4 = Ala;
A^5 = Val;
A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;
A^7 = His;
and, where A^1 is F_5-D-Phe or A^6 is N-methyl-D-Ala, Z_1 is the identifying group of any one of the amino acids Leu, F_5-Phe, or p-X-Phe (where X = H, F, Cl, Br, NO_2, OH or CH_3); provided that where A^1 is other than F_5-D-Phe or A^6 is other than N-methyl-D-Ala, Z_1 can only be the identifying group of F_5-Phe; and each Z_2, Z_3 and Z_4, independently, is H, lower alkyl, lower phenylalkyl, or lower naphthylalkyl; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl.

11. A therapeutic peptide comprising between seven and ten amino acid residues, inclusive, said peptide being an analog of one of the following naturally occurring peptides terminating at the carboxy-terminus with a Met residue: (a) litorin; (b) the ten amino acid carboxy-terminal region of mammalian gastrin releasing peptide; and (c) the ten amino acid carboxy-terminal region of amphibian bombesin; said therapeutic peptide being of the formula:



wherein

A^0 = Gly, Nle, α-aminobutyric acid, or the D-isomer of any of Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal, or is deleted;

A^1 = pGlu, Nle, α-aminobutyric acid, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), F_5-Phe, Trp, Cys, Ser, or β-Nal, or is deleted;

A^2 = pGlu, Gly, Ala, Val, Gln, Asn, Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, β-Nal, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala, Val, Gln, Asn, Gly, Leu, Ile, Nle, α-aminobutyric acid, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H or CH_3), Trp, Cys, or β-Nal;

A^5 = Gln, Asn, Gly, Ala, Leu, Ile, Nle, α-aminobutyric acid, Met, Val, p-X-Phe (where X = F, Cl, Br, OH, H or CH_3), Trp, Thr, or β-Nal;

A^6 = Sar, Gly, Ala, N-methyl-Ala, Val, Gln, Asn,

Leu, Ile, Met, p-X-Phe (where X = F, Cl, Br, NO_2, OH, H, or CH_3), Trp, Cys, or β-Nal;

A^7 = 1-methyl-His, 3-methyl-His, or His;

wherein each Z_{20} and Z_{30}, independently, is H, lower alkyl, lower phenylalkyl, lower naphthylalkyl; further provided that, when either of Z_{20} or Z_{30} is other than H, A^7 is His, A^6 is Gly, A^5 is Val, A^4 is Ala, A^2 is His, and either of R_1 or R_2 is other than H, A^1 is F_5-D-Phe; further provided that either A^1 must be F_5-D-Phe or A^6 must be N-methyl-D-Ala; provided that, if A^0 is present, A^1 cannot be pGlu; further provided that, if A^0 or A^1 is present, A^2 cannot be pGlu; further provided that, when A^0 is deleted and A^1 is pGlu, R_1 must be H and R_2 must be the portion of Glu that forms the imine ring in pGlu; further provided that any asymmetric carbon atom can be R, S or a racemic mixture; and further provided that each R_1 and R_2, independently, is H, C_{1-12} alkyl, C_{7-10} phenylalkyl, COE_1 (where E_1 is C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl), or lower acyl, and R_1 and R_2 are bonded to the N-terminal amino acid of said peptide, and further provided that when one of R_1 or R_2 is COE_1, the other must be H, or a pharmaceutically acceptable salt thereof.

12. The therapeutic peptide of claim 11 wherein

A^0 = Gly, D-Phe, or is deleted;

A^1 = p-Glu, D-Phe, F_5-D-Phe, D-Ala, D-β-Nal, D-Cpa, or D-Asn;

A^2 = Leu, Gln, His, 1-methyl-His, or 3-methyl-His;

A^4 = Ala;

A^5 = Val;

A^6 = Sar, Gly, D-Phe, N-methyl-D-Ala, or D-Ala;

A^7 = His;

and, where each Z_{20} and Z_{30}, is H; and each R_1 and R_2, independently, is H, lower alkyl, or lower acyl; provided that either A^1 must be F_5-D-Phe or A^6 must be N-methyl-D-Ala.

13. The therapeutic peptide of claim 5 of the formula:

p-Glu-Gln-Trp-Ala-Val-Gly-His-statine-amide.

14. The therapeutic peptide of any one of claims 5, 7, 9, and 11 wherein said analog is at least 25% homologous with said naturally occurring peptide.

15. The therapeutic peptide of any one of claims 5, 7, 9, and 11 wherein said analog is at least 50% homologous with said naturally occurring peptide.

16. The therapeutic peptide of claim 7 wherein R_4 is CH_2-NH, and said carbon atom is bonded to Z_2 is of said R configuration.

17. The therapeutic peptide of claim 5 of the formula D-Cpa-Gln-Trp-Ala-Val-Gly-His-β-Leu-NH_2.

18. The therapeutic peptide of claim 5 of the formula

D-Cpa-Gln-Trp-Ala-Val-D-Ala-His-β-Leu-NH_2.

19. The peptide of claim 5 wherein V is OR_4, and R_4 is any of C_{1-20} alkyl, C_{3-20} alkenyl, C_{3-20} alkinyl, phenyl, naphthyl, or C_{7-10} phenylalkyl, and A^6 is N-methyl-D-Ala or A^1 is D-F_5-Phe.

20. The therapeutic peptide of claim 19 of the formula D-Phe-Gln-Trp-Ala-Val-N-methyl-D-Ala-His-Leu-methylester.

21. The therapeutic peptide of claim 19 of the formula D-F_5-Phe-Gln-Trp-Ala-Val-D-Ala-His-Leu-methylester.

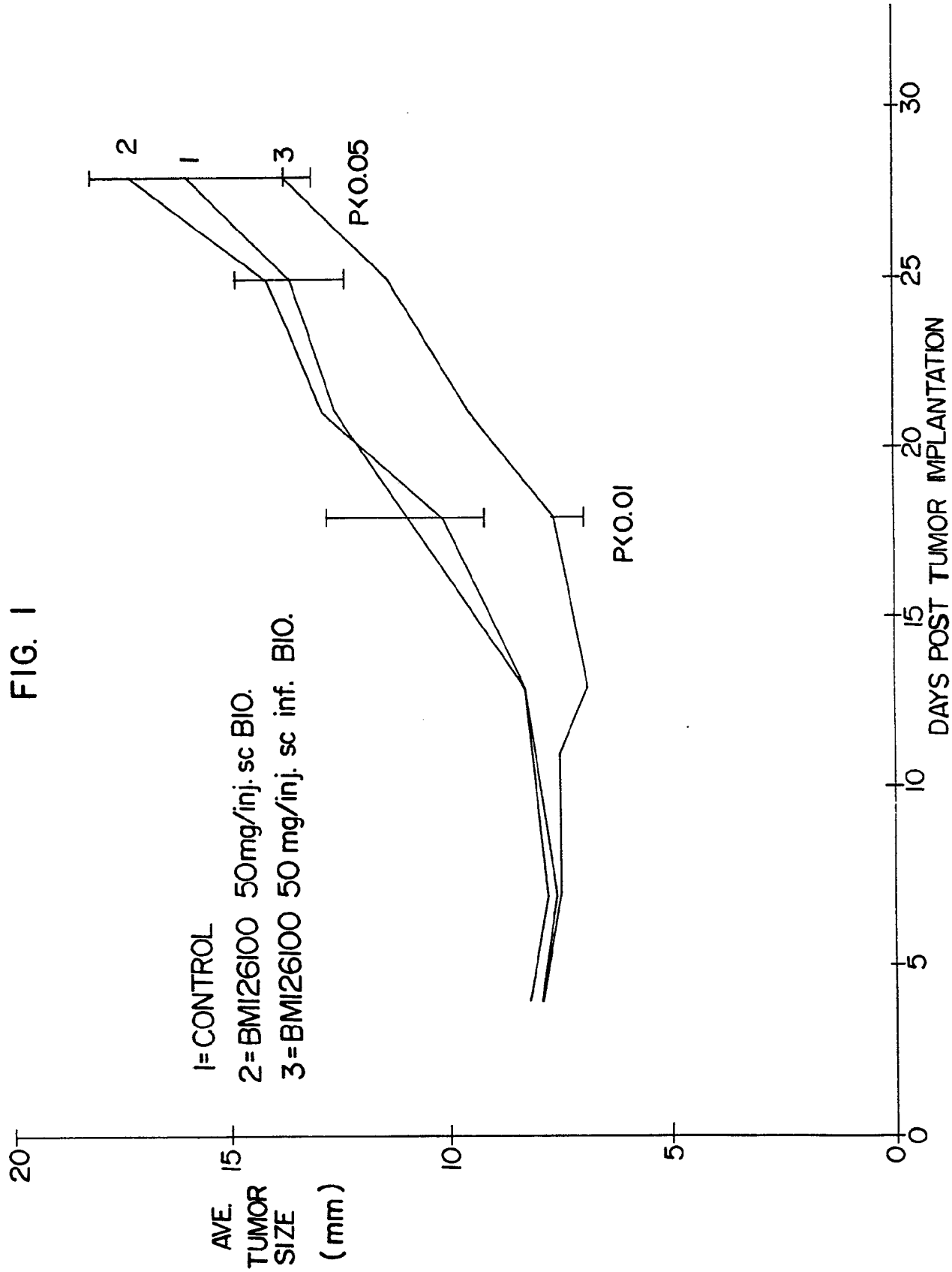
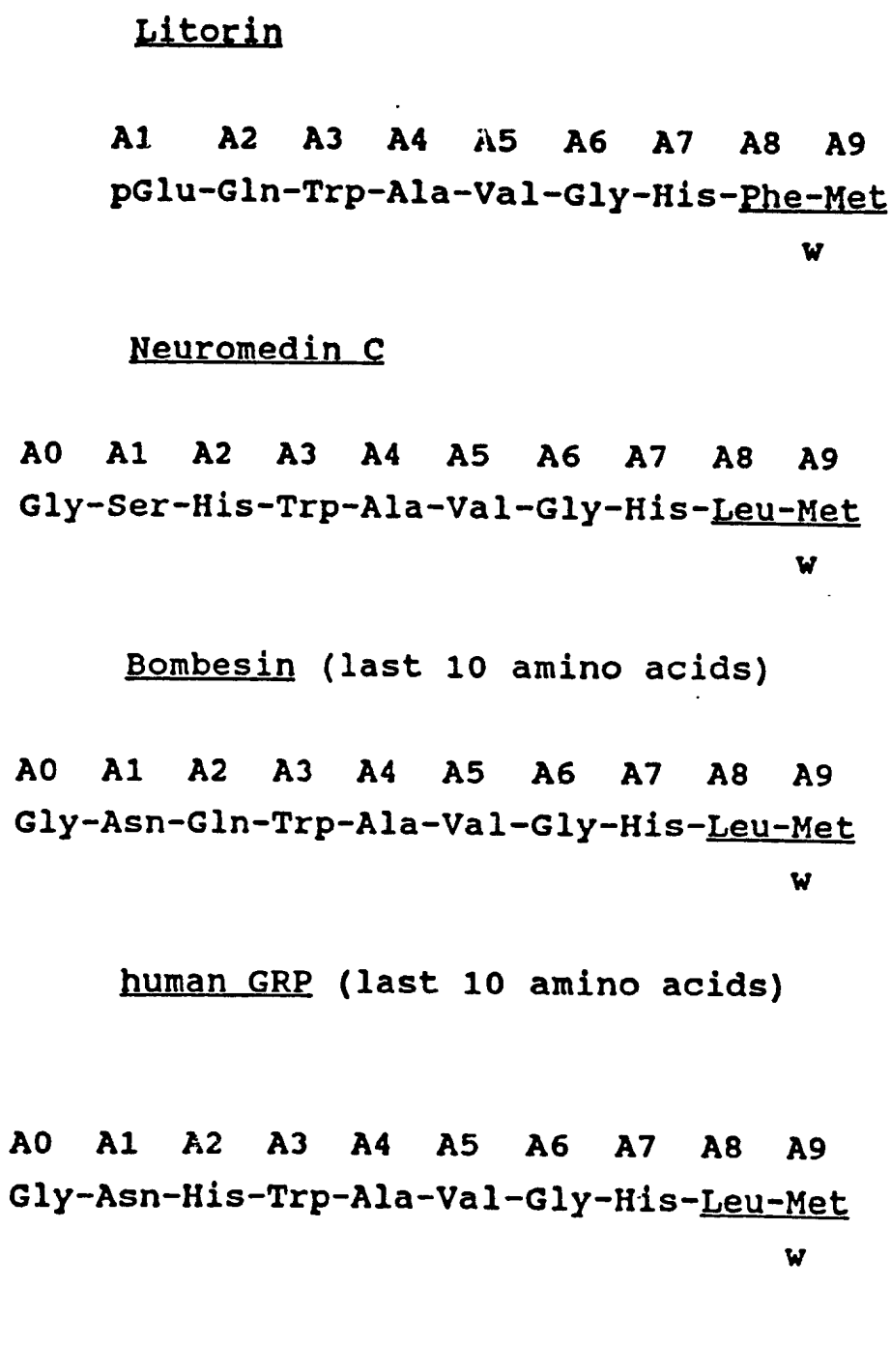

AVE. TUMOR SIZE (mm)
20
15
10
5
0
0
5
10
15
20
25
30
DAYS POST TUMOR IMPLANTATION
1=CONTROL
2=BMI26100 50mg/inj. sc BIO.
3=BMI26100 50 mg/inj. sc inf. BIO.
P<0.05
P<0.01
1
2
3

FIG. 1

FIG. 2


Litorin
A1 A2 A3 A4 A5 A6 A7 A8 A9
pGlu-Gln-Trp-Ala-Val-Gly-His-Phe-Met
W
Neuromedin C
A0 A1 A2 A3 A4 A5 A6 A7 A8 A9
Gly-Ser-His-Trp-Ala-Val-Gly-His-Leu-Met
W
Bombesin (last 10 amino acids)
A0 A1 A2 A3 A4 A5 A6 A7 A8 A9
Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met
W
human GRP (last 10 amino acids)
A0 A1 A2 A3 A4 A5 A6 A7 A8 A9
Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met
W

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04646

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):C07K 5/02,5/06,5/08,5/10,7/02,7/06,7/08,7/10,7/30

U.S.CL.: 530/309,323,324,325,326,327,328,329,330,331,332,345

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	530/309,323,324, 325, 326,327,328,329,330,331,332,345

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstracts and Biological Abstracts Online Computer Search

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,803,261 (COY ET AL.) 07 February 1989. See column 3, lines 7-9 in particular.	1-4,7-8,14-16
Y	The Journal of Biological Chemistry, volume 263, No. 11, issued 15 April 1988, Coy et al., "Probing Peptide Backbone Function in Bombesin", pages 5056-5060. See page 5056 in particular.	1-4 7-8,14-16
Y	Proc. Natl. Acad. Sci. USA, volume 82, issued November 1985, Zachary et al., "High-affinity receptors for peptides of the bombesin family in Swiss 3T3 cells" pages 7616-7620. See Table 2 in particular.	7-8,14-16

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
25 September 1990	10 JAN 1991
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	Christina Chan CHRISTINA CHAN